# In This Issue

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## "TIL DEATH DO US PART" – KERATINOCYTES DYING TO LEAVE THE EPIDERMIS

I suspect that no matter how many times readers of this journal have attended wedding ceremonies in which these words were spoken, absolutely no one ever thought about the biology of epidermis. However, in a somewhat ironic twist of fate, we as investigative skin biologists know our survival beyond the womb and wedding days of our lives is critically dependent on a thin layer of dead keratinocytes (KCs) that form the stratum corneum. This barrier function of skin, our so-called protective shield, reflects a complex and poorly understood series of biochemical events in which a balance is achieved between cellular proliferation, differentiation, and death. These processes are highly interconnected, and the regulatory mechanisms are beginning to be understood at the molecular level. Thus, for us to avoid death from infection or desiccation, our epidermal KCs must first undergo terminal differentiation prior to cell death, followed by departing or desquamating from the skin surface. By following the ultimate cell fate pathway – cell death, KCs in the stratum granulosum give rise to the stratum corneum.

In this issue of the *JID*, there are several reports providing important insights into the complex process regulating KC proliferation, differentiation, and cell death, which have implications for several skin diseases including psoriasis. In addition, a few authors also tackle a somewhat related issue, which involves the ability of those KCs that are not dying to leave the epidermis, but instead these living KCs produce inflammatory mediators that contribute to innate immune responses of skin. These latter studies remind us of the versatility of KCs, not only contributing to the brick and mortar foundation of skin, but also as active and dynamic immunocytes capable of initiating vitally important inflammatory responses by secretion of cytokines regulated by transcriptional factors such as NF-κB. Of course, as many adolescents can attest, sometimes these inflammatory reactions become dysregulated, giving rise to acne. In the third and final section of this overview, several articles dealing with stress and skin disorders are discussed.

When skin is exposed to excessive sunlight, epidermal KCs are diverted from the physiological "planned cell death pathway" and undergo premature apoptosis, creating so-called "sun-burn" cells predominantly located in the mid and lower level layers of epidermis. Using primary normal human KCs, in culture, Pelle *et al* (p. 177) report that exposure to UVB light triggers oxidative DNA damage characterized by increased 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG) residues. Such oxidative DNA damage was significantly reduced by pretreating KCs with mannitol. The generation of DNA damage could also be reduced by addition of catalase, implicating a role for $H_2O_2$. The authors postulate that UVB irradiation promotes $H_2O_2$ migration from cytosol to nucleus, with an ensuing Fenton-like reaction producing reactive hydroxyl radicals mediating the 8-oxo-dG formation of cellular DNA adducts. This work is significant because it provides yet another mechanism (oxidative stress) by which UV-light can influence the biology of KCs, to supplement previously established mechanisms which involved: alterations in the plasma membrane with oligomerization of death receptors; alterations in mitochondrial function; and DNA damage caused by thymine dimers and other UV-light mediated signature mutations in DNA. Understanding these mechanistic pathways that facilitate removal of KCs bearing genetic alterations is of oncological significance given the strong links between sun exposure and skin cancer. From a clinical perspective, it may be difficult to achieve a favorable therapeutic outcome by which KCs bearing UV-light induced mutations are selectively removed without adversely influencing the delicate balance that maintains the barrier function of skin.

Using a chemical based system for examining the stress-induced cytotoxic response of KCs, Bakondi *et al* (p. 88) characterized immortalized cells (i.e., HaCaT cells) before and after exposure to a calcium chelator, and upon reaching confluency. Addition of the nitric oxide-derived cytotoxic mediator-peroxynitrite triggers an influx of calcium ions and death of HaCaT cells. If a cell permeable calcium chelator is added, significant cytoprotection against peroxynitrite and $H_2O_2$ mediated cell killing is achieved. Moreover, when comparing rapidly proliferating HaCaT cells to cells that have reached confluency, achievement of high cell density also protected the cells from the cytotoxicity of various agents. The mechanisms for this death defying phenotype were correlated to an inhibition of various effector caspase pathways. The authors suggest that these experimental in-vitro findings using HaCaT cells may provide insight into the clinical observation that superficial, highly differentiated KCs are relatively more resistant to oxidative stress compared to proliferating basal layer KCs. These findings also highlight the conundrum alluded to in the opening paragraph. On one hand, the most differentiated KCs in the upper layers of epidermis are expected to be highly resistant to cell death. Yet, on the other hand, within one cell layer throughout the entire integumentary system, KCs in the stratum granulosum die to create corneocytes. What a marvelous mystery to try and unravel that occurs every second of every day right in front of our eyes!

Moving from UV-light and peroxynitrite-mediated stimuli for triggering premature cell death in epidermal cells, Leverkus *et al* (p. 149) probe the relative contributions of specific death receptors in the apoptotic response of HaCaT cells. By employing selective blocking antibodies, these authors report the apoptotic response of HaCaT cells to a recombinant leucine zipper form of TRAIL (i.e., LZ-TRAIL) was mediated predominantly by the TRAIL-R1 type of receptor expressed on the surface of HaCaT cells. Interestingly, not only does TRAIL exposure induce apoptosis, it could also activate NF-κB signaling with enhanced production of cytokines such as IL-8. In this regard, TRAIL resembles another family member, TNF-α, which can also provoke inflammatory cytokine release from KCs. The authors speculate that if cell death pathways are inhibited, TRAIL-mediated signaling elicits pro-inflammatory responses by epidermal KCs in which chemotactic pathways and a cytokine release could play

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important roles in the skin immune system. Of course, in diseases like psoriasis in which epidermal KCs acquire an apoptotic resistant phenotype, such NF-κB signaling by TNF family members may well be important pathways contributing to the pathophysiology of the disease process characterized by persistent inflammation.

In the fourth article in this series, Cerezo *et al* (p. 110) also used HaCaT cells to explore the tight balance between cell proliferation and terminal differentiation. By directly or indirectly (via c-myc) activating telomerase, the authors report that hTert/telomerase appears to play a significant role in regulating epidermal differentiation. Telomerase activity has been previously studied in the context of prolonging longevity of normal and neoplastic cells, and has been observed to correlate closely with proliferation. This report suggests that down-regulation of telomerase activity during terminal differentiation is not a mere consequence, but rather an active participant in the differentiation process itself. Using organotypic cocultures, HaCaT cells engineered to overexpress hTert where found to be lacking in both filaggrin and loricrin proteins, which are key components of terminally differentiated epidermal cells. Thus, it appears forced TERT/telomerase expression confers a phenotypic function besides telomerase length regulation that could influence terminal differentiation and stratum corneum production in the epidermis. Whether the impaired terminal differentiation characteristically presents within psoriatic plaques is related to the increased telomerase activity of psoriatic KCs remains to be determined.

## CYTOKINE NETWORKING, ACNE, AND INNATE IMMUNITY

Even though acne vulgaris is relatively common, the precise inflammatory pathway responsible for generating and perpetuating papules and pustules remains to be defined. While dissection of the cytokine network in acne has lagged behind the psoriasis field, rapid progress is now being made. Holland *et al* (p. 20) report that specific inflammatory events are taking place in even the very earliest stages of acne lesion development. Using immunohistochemistry and quantitative assessments, the authors document increased numbers of $CD4^+$ T cells and macrophages in early lesions, accompanied by vascular endothelial cells expressing various adhesion molecules. A prominent increase in IL-1α positive cells was observed in acute acne lesions, and the authors speculate that high sebum production could perturb the barrier formation of the follicular unit leading to release of IL-1α and TNF-α that initiate an inflammatory cascade accompanied by recruited $CD4^+$ memory T cells and macrophages. By demonstrating these cellular and cytokine changes in early lesions, the authors argue that acne should be classified as an inflammatory skin disease, shifting emphasis away from the hypercornification of the follicular wall as a primary event in the pathogenesis of acne. If this argument sounds familiar, it should because psoriasis investigators have shared a similar dialogue for decades in which the pendulum has swung between those investigators that favored a primary KC basis versus those investigators favoring a T cell basis for this common and enigmatic disease. Perhaps the use of novel anti-inflammatory agents (TNF inhibitors) or drugs that selectively target T cells in acne patients will help resolve this fundamentally important issue.

In the Letters to the Editor section, there is an exchange between two laboratories dealing with the expression and function of Toll-like receptors by human KCs. With the increasing awareness of innate immunity in dermatology, the Toll-like receptor expression patterns have begun to be dissected by several groups, and a receptor for the bacterial-derived lipopdysaccharide (LPS) has been a frequent target for investigators. One group (Song *et al*) provided evidence that KCs can respond to LPS by rapid intracellular calcium flux, NF-κB activation, and cytokine release via TLR4. However, another group (Kawai *et al*) does not believe KCs express functional TLR4, implicating instead TLR2. As specific molecular components of the TLR family are studied on KCs, Langerhans cells, and other resident and recruited cell types in skin, the relatively new field of innate immunity in the context of cutaneous immunology and inflammation are likely to yield insights into many skin diseases such as acne and psoriasis, to name a few. Here is one recent example of relevance to dermatology: polymorphic alleles for TLR2 are now associated with lepromatous leprosy, which correlate to impaired NF-κB mediated signaling and poor cellular immune responses characteristic of Hansen's disease.

## STRESS AND SKIN DISORDERS

Many skin diseases are influenced by psychological stress. Compared to the articles mentioned above dealing with sunlight and chemical-induced stress responses in human KCs, the article by Inoue *et al* (p. 165) explores the relationship between melanocyte-mediated pigmentation and stress in mice. Stress was induced by increasing the population density or by restraining mice, which produced elevated plasma and skin ACTH levels. Following UVB-irradiation, stressed mice developed tanning with increased numbers of DOPA-positive melanocytes. While it is unclear exactly how the pigmentary response was augmented under conditions of stress, this report may come to mind the next time one leaves the bench to go to the beach. It would seem that the more crowded the beach, the better the chances of acquiring a tan. However, as noted in the next article, if excessive sun exposure produces a melanoma, knowledge of protein kinase C (PKC) signaling will come in more handy than a beach umbrella.

There are more than 10 PKC isoforms identified in mammalian tissues. In the report by Oka *et al* (p. 69), a key role for PKC-α and its association with phospholipase D activity is highlighted in human melanoma cells. These investigators provide evidence that up-regulation of PKC-related signaling elements play important roles in melanoma progression. It will be of interest to determine if targeting this pathway will have any therapeutic effect in patients with melanoma.